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BENZOQUINONES FROM *EMBELIA ANGUSTIFOLIA*

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Key Word Index—*Embelia angustifolia*; Myrcinaceae; 2,5-dihydroxy-1,4-benzoquinones; embelin; angiotensin-converting enzyme; ACE.

Abstract—Three new 2,5-dihydroxy-3-alkyl-1,4-benzoquinones, (*Z*)-2,5-dihydroxy-3-(pentadec-8-enyl)-1,4-benzoquinone, (*Z,Z*)-2,5-dihydroxy-3-(heptadeca-8,11-dienyl)-1,4-benzoquinone and (*Z*)-2,5-dihydroxy-3-(heptadec-8-enyl)-1,4-benzoquinone and the known 2,5-dihydroxy-3-pentadecyl-1,4-benzoquinone were isolated from the leaves of *Embelia angustifolia*. Their structures have been established on the basis of spectral analysis and by chemical methods. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

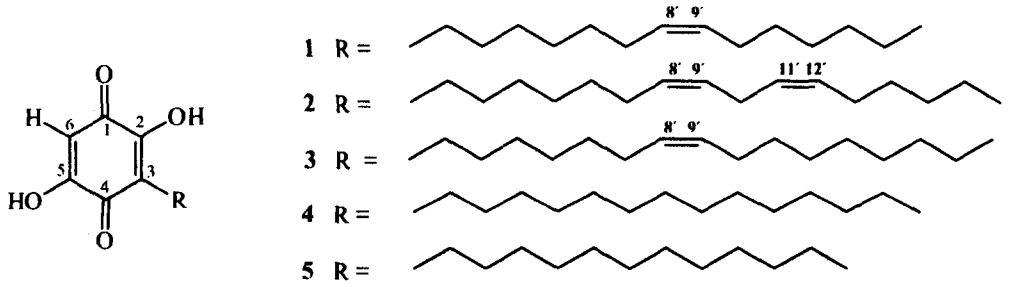
Embelia angustifolia (vernacular name *Liane savon*) is endemic to Réunion Island and Mauritius and is used locally in the treatment of urinary disorders, nephritis, cysts, colic and rheumatism [1]. In view of the fact that the diuretic effect might be related to angiotensin-converting enzyme (ACE) inhibition, a bioassay-directed fractionation of the ether-soluble components of the dried plant was undertaken, the *in vitro* ACE inhibitory activity being determined as described by Elbl and Wagner [2] and later modified by Hansen *et al.* [3]. This report deals with the isolation and structure elucidation of four ACE inhibitory 2,5-dihydroxy-3-alkyl-1,4-benzoquinones of which three are new compounds.

RESULTS AND DISCUSSION

The four ACE inhibitors (**1–4**) from *E. angustifolia* were isolated collectively by silica gel chromatography but were readily separated by reversed phase HPLC on macroporous polystyrene (PLRP-S).

Compounds **1–4** are brick red and their UV spectra show the characteristic pattern for a 2,5-dihydroxy-1,4-benzoquinone, with absorption at about 288 nm and 417 nm [4]. The IR spectra are also characteristic of 2,5-dihydroxy-1,4-benzoquinones, with bands at about 3305 (OH) and 1610 (>C=O) cm^{-1}. This structural relationship was confirmed by comparison with authentic embelin (**5**) which is a common constituent in the family Myrcinaceae [5, 6]. It is noteworthy that **5** showed strong ACE inhibitory activity in the bio-assay employed.

The EI mass spectra of **1–4** were similar to that of **5**, pointing to 2,5-dihydroxy-1,4-benzoquinone structures, each with a single side chain at position 3. This was evident from the occurrence in each case of a strong peak at *m/z* 154, formed by elimination of the side chain in a McLafferty rearrangement [7]. Differences in M_r, as observed from strong parent peaks, could then be related to differences in the number of carbon atoms and in the number of double bonds in these side chains.



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Confirmation of this was found in the ^{1}H NMR spectra of **1–4**, from which the side chains were determined to be unbranched. Thus compounds **1**, **2** and **3** possessed pentadecenyl-, heptadecadienyl- and heptadecenyl- side chains, respectively, whereas **4**, with a saturated pentadecyl side chain was the known compound homorapanone [8].

The configuration of the double bonds in **1–3** was established by means of ^{1}H-^{13}C-correlated NMR spectra. In each case the δ-values for allylic carbons were found to be about 27 ppm. This corresponds to *cis* double bonds in an unbranched chain [9]. In the case of *trans* configurations 6–7 ppm higher δ-values would have been expected.

The relative position of the two double bonds in the side chain of **2** was determined by a comparison with (*Z*,*Z*)-9,12-octadecadienoic acid (linoleic acid). In both cases the ^{1}H NMR spectra show a pattern corresponding to the sequence —CH=CH—CH_2—CH=CH— with the methine groups at δ 5.3 and the methylene group as a triplet at δ 2.8.

The exact placement of the double bonds in the side chains was established by epoxidation followed by periodate cleavage [10]. The aldehydes formed from the terminal ends of the chains were then identified by GC-MS. In this way **1** was shown to be (*Z*)-2,5-dihydroxy-3-(pentadec-8-enyl)-1,4-benzoquinone, **2** was (*Z*,*Z*)-2,5-dihydroxy-3-(heptadeca-8,11-dienyl)-1,4-benzoquinone, and **3** was (*Z*)-2,5-dihydroxy-3-(heptadec-8-enyl)-1,4-benzoquinone.

EXPERIMENTAL

^{1}H and ^{13}C NMR: 200 MHz and 50.3 MHz respectively. Multiplicities in ^{13}C NMR spectra have been deduced from DEPT spectra; CC: Silica gel-KH_2PO_4 containing 6.2% KH_2PO_4 and 3% water. UV: MeOH–conc. HCl (999:1); GC-MS: HP5890 Series II gas chromatograph directly coupled to a Jeol JMS-AX505W mass spectrometer: SGE BPX5 column (25 m × 0.25 mm, 0.25 μm film thickness), oven temp programme 50° for 2 min, 50 to 200° at 5°/min, 200° for 10 min, head pressure 70 kPa, injection temp 250° with split injection. The spectrometer repetition rate was 1.0 scan/sec. The ion source was run in EI mode at 240°, 70 eV ionization energy. Identification of aldehydes was done by comparison of acquired mass spectra with spectra of the instruments library (NIST) and for hexanal also by comparison of the R_t with that of an authentic sample.

Plant material. Leaves of *Embelia angustifolia* (A.DC) DC in DC, were collected in February 1994, at Bélouve at Réunion Island in the Indian Ocean. A voucher specimen (A. & H. Adsersen, 5433) is deposited in Herbarium C., Botanical Museum, Copenhagen, Denmark.

Extraction and isolation. The dried and powdered plant material (515 g) was extracted in a Soxhlet apparatus for 6 hr with Et_2O. The extract was partitioned in the system pentane–MeOH–H_2O (10:9:1) and the polar phase evapd. CC of the residue (3.4 g) on silica gel–KH_2PO_4 with CH_2Cl_2–EtOAc (9:1) → (4:1) afforded a mixture of **1–4** (0.71 g) which was separated by rev. phase prep. HPLC on macroporous polystyrene (PLRP-S) with AcOH–H_2O–CH_4Cl_2–MeCN (2:27:5:66) as eluent. Yields (given in order of elution from PLRP-S): **1** 40 mg, **2** 150 mg, **3** 10 mg and **4** 50 mg. The separated compounds were finally purified by CC on macroporous polystyrene, XAD-2 with MeOH–CH_2Cl_2–H_2O–AcOH (180:9:9:2) as eluent.

(Z)-2,5-*Dihydroxy*-3-(*pentadec*-8-*enyl*)-1,4-*benzo-quinone* (**1**). Orange crystals (92% MeOH); mp 116–119°; UV $\lambda_{max}^{MeOH-HCl}$ nm (log ε): 288.0 (4.33) and 418.8 (2.48); IR ν_{max}^{KBr} cm^{-1}: 3305 (OH), 1610 (>C=O) and 1330 (C—O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.69 (2H, *br*, OH), 6.01 (1H, *s*, H-6), 5.35 (2H, *m*, H-8′, H-9′), 2.45 (2H, *t*, J = 7.4 Hz, H-1′), 2.03 (4H, *m*, H-7′ and H-10′), 1.46 and 1.28 (18H, *m*, H-2′ and H-3′ to H-6′, H-11′ to H-14′ resp., overlapping), 0.89 (3H, *t*, J = 7 Hz, H-15′); ^{13}C NMR ($CDCl_3$): δ *ca.* 168 (very *br* C-1, C-2, C-4, C-5), 129.9 (*d*, C-8′, C-9′), 117.0 (*s*, C-3), 102.2 (*d*, C-6), 32.0 (*t*, C-13′), 29.8–29.3 overlapping (triplets, C-3′ to C-6′, C-11′, C-12′), 27.9 (*t*, C-2′), 27.2 (*t*, C-7′*), 26.9 (*t*, C-10′*), 22.5 (*t*, C-1′), 22.4 (*t*, C-14′), 14.0 (*q*, C-15′), assignments marked * are exchangeable; EIMS 70 eV *m/z* (rel. int.): 348 $[M]^+$ (90), 154 $[M-C_{14}H_{26}]^+$ (100).

(Z,Z)-2,5-*Dihydroxy*-3-(*heptadeca*-8,11-*dienyl*)-1,4-*benzoquinone* (**2**). Orange crystals (92% MeOH); mp 91–97°; UV $\lambda_{max}^{MeOH-HCl}$ nm (log ε): 287.0 (4.32) and 415.0 (2.52); IR ν_{max}^{KBr} cm^{-1}. 3300 (OH), 1610 (>C=O) and 1330 (C—O) cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 7.71 (2 H, *br*, OH), 6.01 (1H, *s*, H-6), 5.37 (4H, *m*, H-8′, H-9′, H-11′, H-12′), 2.77 (2H, *br t*, J = 5.5 Hz, H-10′), 2.45 (2H, *t*, J = 7.4 Hz, H-1′), 2.03 (4H, *m*, H-7′ and H-13′), 1.47 (2H, *m*, H-2′) overlapping with 1.31 (14H, *m*, H-3′ to H-6′ and H-14′ to H-16′), 0.89 (3H, *t*, J = 7 Hz, H-17′); ^{13}C NMR ($CDCl_3$): δ *ca* 170 (*br*, C-1, C-2, C-4, C-5), 130.2, 130.1, 128.0 and 127.9 (*d*, C-8′, C-9′, C-11′, C-12′), 116.9 (*s*, C-3), 102.2 (*d*, C-6), 31.5 (*t*, C-15′), 29.6–29.2 overlapping (triplets, C-3′ to C-6′ and C-14′), 27.9 (*t*, C-2′), 27.2 (*t*, C-7′, C-13′), 25.6 (*t*, C-10′), 22.6 (*t*, C-16′), 22.5 (*t*, C-1′), 14.1 (*q*, C-17′); EIMS 70 eV *m/z* (rel.int): 374 $[M]^+$ (75), 154 $[M-C_{16}H_{28}]^+$ (100).

(Z)-2,5-*Dihydroxy*-3-(*heptadec*-8-*enyl*)-1,4-*benzo-quinone* (**3**). Orange crystals (92% MeOH); mp 111–112°; UV $\lambda_{max}^{MeOH-HCl}$ nm (log ε): 288.2 (4.32) and 415.8 (2.51); IR ν_{max}^{KBr} cm^{-1}. 3305 (OH), 1615 (>C=O) and 1330 (C—O) cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 7.72 (2H, *br*, OH), 6.01 (1H, *s*, H-6), 5.35 (2H, *m*, H-8′, H-9′), 2.45 (2H, *t*, J = 7.5 Hz, H-1′), 2.02 (4H, *m*, H-7′ and H-10′), 1.47 (2H, *m*, H-2′) overlapping with 1.30 (20H, *m*, H-2′ to H-6′ and H-11′ to H-16′), 0.88 (3H, *t*, J = 7 Hz, H-17′); ^{13}C NMR ($CDCl_3$): δ *ca* 170 (*br*, C-1, C-2, C-4, C-5), 130.0 (*d*, C-8′*), 129.8 (*d*, C-9′*), 117.0 (*s*, C-3), 102.2 (*d*, C-6), 31.9 (*t*, C-15′), 29.8–29.2 overlapping (triplets, C-3′ to C-6′, C-11′ to C-14′), 27.9 (*t*, C-2′), 27.2 (*t*, C-7′*), 27.2 (*t*, C-10′*), 22.7 (*t*, C-16′),

22.5 (*t*, C-1′), 14.1 (*q*, C-17′), assignments marked * are exchangeable; EIMS 70 eV *m/z* (rel.int): 376 $[M]^+$ (100), 154 $[M-C_{16}H_{30}]^+$ (92).

2,5-Dihydroxy-3-pentadecyl-1,4-benzoquinone (**4**). Not crystallized $C_{21}H_{34}O_4$; ^1H NMR ($CDCl_3$/DMSO-d_6 (2:1)): δ 5.76 (1H, *s*, H-6), 2.34 (2H, *t*, $J = 7.0$ Hz, H-1′), 1.25 (26H, *m*, H-2′ to H-14′), 0.87 (3H, *t*, $J = 6$ Hz, H-15′); ^{13}C NMR ($CDCl_3$/DMSO-δ_6 (2:1)): δ 117.5 (C-3), 103.5 (C-6), 31.5 (C-13′), 29.3–27.8 overlapping (C-2′ to C-12′), 22.3 (C-1′*), 22.2 (C-14′*), 14.0 (C-15′), assignments marked * are exchangeable; EIMS 70 eV *m/z* (rel.int): 350 $[M]^+$ (100), 154 $[M-C_{14}H_{28}]^+$ (100).

Epoxidation of **1**. A mixture of **1** (4.9 mg), 70% 3-chloro-perbenzoic acid (5.1 mg), and $CHCl_3$ was left to stand at room temp for 9 hr. 30 μl of a 10% dimethylsulphide soln in MeOH was added to stop the reaction. The solvent was removed *in vacuo* and the residue chromatographed by rev. phase. prep. HPLC on PLRP-S with AcOH–water–CH_2Cl_2–MeCN (2:34:3:61) to yield epoxide **6** (3.6 mg).

Epoxide **6**. ^1H NMR ($CDCl_3$): δ 6.01 (1H, *s*, H-6), 2.92 (2H, *m*, H-8′, H-9′), 2.45 (2H, *t*, $J = 7.4$ Hz, H-1′), 1.49 (6H, *m*, H-2′, H-7′, H-10′) overlapping with 1.41–1.30 (16H, *m*, H-3′ to H-6′, H-11′ to H-14′), 0.92 (3H, *t*, $J = 7$ Hz, H-15′).

Cleavage of epoxide **6**. Epoxide **6** (0.18 mg) was dissolved in dry $CHCl_3$ (275 μl) and added to finely powdered HIO_2 (0.6 mg). The reaction mixture was stirred at 40° for 3 hr and then subjected to GC-MS. The aldehyde produced from the terminal end of the side chain was resolved and identified as heptanal by its MS.

Epoxidation of **2**. A mixture of **2** (12.1 mg) was epoxidized approximately as described for **1**. Rev. phase. prep. HPLC on PLRP-S with AcOH–water –CH_2Cl_2–MeCN (4:80:5:111) yielded epoxide (**7**) (6.8 mg).

Epoxide **7**. ^1H NMR ($CDCl_3$): δ 6.01 (1H, *s*, H-6), 3.12 and 3.00 overlapping (4H, *m*, H-8′, H-9′, H-11′, H-12′), 2.45 (2H, *t*, $J = 7.3$ Hz, H-1′), 1.76 (2H, *m*, H-10′), 1.51 (6H, *m*, H-2′, H-7′, H-13′) overlapping with 1.35 (14H, *m*, H-3′ to H-6′, H-14′ to H-16′), 0.9 (3H, *t*, H-17′); ^{13}C NMR ($CDCl_3$): mixture of diastereomers a and b, (2:1); δ 116.8 (C-3), 102.2 (C-6), 57.1 and 57.1 (C-9′, C-11′ a), 56.9 and 56.8 (C-9′, C-11′ b), 54.4 (C-8′, C-12′ a), 54.3 (C-8′, C-12′ b), 31.7 (C-15′), 29.3–27.8 overlapping (C-2′ to C-6′, C-14′), 27.2 (C-10′ a), 26.9 (C-10′ b), 26.4 and 26.1 (C-7′, C-13′ a), 26.5 and 26.3 (C-7′, C-13′ b), 22.6 (C-16′*), 22.4 (C-1′*), 14.0 (C-17′), assignments marked * are exchangeable.

Cleavage of epoxide **7**. Method as described for **6**. The aldehyde produced from the terminal end of the side chain was identified as hexanal.

Epoxidation of **3**. (5.1 mg) of **3** was epoxidized and subjected to HPLC as described for **1** to yield epoxide (**8**) (4.0 mg).

Epoxide **8**. ^1H NMR ($CDCl_3$): δ 5.96 (1H, *s*, H-6), 2.91 (2H, *m*, H-8′, H-9′), 2.44 (2H, *t*, H-1′), 1.48 (6H, *m*, H-2′, H-7′, H-10′) overlapping with 1.27 (20H, *m*, H-3′ to H-6′, H-11′ to H-16′) and 0.88 (3H, *t*, H-17′).

Cleavage of epoxide **8**. Method as described for **6**. The aldehyde produced from the terminal end of the side chain was identified as nonanal.

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